Ceriodaphnia dubia 7-Day Chronic Toxicity Test

1.0 OBJECTIVE

In laboratory tests designed to determine the toxicity of low salinity water samples, *Ceriodaphnia* neonates are exposed to test solutions for 7 days, after which the survival and reproduction is determined in each toxicant concentration. Observed effects may be related to the presence of contaminants or to naturally occurring factors. In order to correctly interpret toxicity results, concentrations of chemical contaminants should be analyzed, as well as other water quality parameters, such as pH, dissolved oxygen, total dissolved solids, hardness, salinity, temperature, ammonia and conductivity.

In this procedure, water samples collected from field stations are divided into replicate beakers in the laboratory. Single *Ceriodaphnia* neonates are placed into each replicate container and monitored for mortality and fecundity. After a 7-day exposure, daily survival and reproduction are used to give an estimate of sample toxicity. Because the test measures effects on an early life-stage of an ecologically important species possessing relatively stringent water quality requirements, the results constitute a good basis for decisions concerning either hazard evaluation or the suitability of estuarine waters for aquatic life (US EPA 1994).

2.0 EQUIPMENT

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (SOP).

2.1 Culture

- Airstones and clean air system
- 2-liter beakers or similar volume containers
- 4:1 water prepared from Perrier® or Evian® and distilled water (25 ± 1°C.)
- YCT and *Selenastrum* for feeding, purchased from Aquatic Biosystems
- Disposable plastic pipettes (for handling animals)

2.2 Test Initiation

- 30-ml disposable plastic cups (10 per sample concentration, leached in distilled water)
- 1000-mL volumetric flasks (2) and pipettes for reference toxicant dilutions
- Water bath or environmental chamber
- Data sheets
- Gloves and appropriate safety gear (see MPSL lab safety manual)

- Sample vials for reference toxicant analysis (new polyethylene 30 ml, acid washed)
- Graduated pipettes: 1- and 10-ml
- Analytical balance
- Plastic squirt bottles

2.3 Water Quality

- Meters and probes for measuring pH, dissolved oxygen, hardness, ammonia, and conductivity
- Thermometers (glass mercury thermometer and continuously recording thermometer)
- Graduated pipettes (10-ml) and hand pipette pump for water quality sampling
- Gloves and appropriate safety gear (see MPSL lab safety manual)

2.4 DILUTION WATER

In every step of this procedure, use Granite Canyon Nanopure® water mixed with Evian® in a 4:1 ratio. Conductivity should not exceed 3000μS at any time. Temperature for *Ceriodaphnia* tests should be 25 ± 1°C. Hardness (as $CaCO_3$) should not exceed 700 mg/liter.

3.0 EXPERIMENTAL DESIGN

Aquatic toxicity tests can be used as screening tools or as part of more comprehensive studies to assess water quality. Careful consideration must be given to site characteristics, reference site selection, field replication, choice of synoptic measures, seasonal factors, and comprehensive planning and peer review to determine that study designs are adequate to meet program objectives.

This laboratory toxicity test consists of ten replicate test cups for each sample concentration. The quality of test animals and testing conditions is determined through concurrent testing of reference toxicants (positive controls) and control water (negative controls). Testing of reference sites or receiving water is recommended to demonstrate the suitability of test sites in the absence of toxic contaminant concentrations. Test conditions of temperature and photoperiod are controlled as indicated below, and pH, NH_3, conductivity, and dissolved oxygen are measured at the beginning and end of the exposure. Temperature is measured continuously, and hardness is measured at the beginning of the test.

4.0 PREPARATION OF SAMPLES FOR TESTING

Because of the short holding time, tests will generally be initiated on the same day as sample receipt. Place appropriate sample volume in the constant temperature room. Allow particulates to settle out and

oxygen concentrations to equilibrate below super-saturated levels. Prepare ten replicate 30-mL cups for each sample to be tested. Each container receives 15 mL of test solution.

5.0 CONTROLS

5.1 Dilution and Conductivity Controls

There should be two dilution controls: one consisting of 4:1 culture water, and another that matches the highest conductivity. If samples are diluted because of high conductivity, the high conductivity control should be prepared to reflect this dilution. Prepare the conductivity control by starting with 4:1 culture water and adding 1-µm filtered seawater dropwise until the initial conductivity is reached. Dilute back to the final conductivity with 4:1 culture water.

5.2 Reference Toxicant Tests

A reference toxicant test must be conducted concurrently with every test to indicate the sensitivity of the organisms and the suitability of the test methodology. Reagent grade copper chloride ($CuCl_2$) should be used as the reference toxicant for *Ceriodaphnia* tests, unless another toxicant is specified by the Regional Water Quality Control Board or other appropriate regulatory agency. Prepare a 10,000 µg/liter $CuCl_2$ stock solution by adding 0.0268g reagent grade $CuCl_2$ to one liter of distilled water in a volumetric flask. Cap tightly and mix thoroughly. Sample the reference toxicant stock solution at the beginning of the test for chemical verification of the copper concentration. Acidify samples for analysis in clean sample vials with 1% by volume 14N-reagent grade nitric acid.

Reference toxicant solutions should be two to five replicates of 0 (control) 5.6, 10, 18, 32, and 56 µg/liter. Other concentrations may be added between these if greater precision is desired for quality control chart purposes. Prepare concentrations according to dilution schedule. Start with the control solutions and progress to the highest concentration to minimize contamination.

All tests (sample and reference toxicant) must use neonates from the same culture. They must be handled in the same way and delivered to the test containers at the same time.

6.0 TEST ORGANISMS

6.1 Culturing *Ceriodaphnia* and Isolating Neonates

The water flea, *Ceriodaphnia dubia*, occurs in littoral areas of lakes, ponds, and marshes throughout most of the world. *Ceriodaphnia* sensitivity to contaminants and their ease of laboratory culture make them suitable organisms for determining the toxicity of chemical compounds, complex effluents, and fresh waters. *Ceriodaphnia* are kept in mass culture in the laboratory in large beakers. Every two weeks 50

neonates are added to one liter of 4:1 water and fed one capful of YCT and *Selenastrum* per day. Cultures are transferred to new 4:1 water twice per week. Individual cultures should be started one week prior to testing to insure neonate availability. Individual cultures are started by placing individual neonates in 30-mL cups with 15 mL 4:1 water. These animals are transferred to new water three times per week, discarding the neonates, and fed at the time of renewal. On day 7 the animals should produce their third broods and there should be an adequate number of neonates for test initiation.

6.2 Loading of Neonates

To load animals into one test board, randomly choose ten brood cups, each with 8 or more neonates, from the individual cultures. Transfer neonates from the first brood cup into the first replicates of the board (the first row). Using a 2-mm bore plastic disposable pipette to transfer the neonates. Maintain water temperature (25°C ± 1) by sorting animals in the constant temperature room where the test is being held.

7.0 DAILY TEST MONITORING AND RENEWAL

7.1 Survival, Brood Size and Transfer

Live animals are transferred to new test solution daily. At the time of transfer survival and brood size are determined and recorded. Aliquot new test solutions to a new set of 30-mL cups and place in the constant temperature room. Begin the renewal when the sample temperatures equilibrate. Set up the workstation for the renewal by placing a dissecting microscope on a light table. Place the old test board on the left of the scope and the new test board on the right. Remove a single cup from the old test board and its corresponding cup from the new board. Place the cups on the light table.

Immobile *Ceriodaphnia* that do not respond to a stimulus are considered dead. The stimulus should be a gentle stream of water from a disposable transfer pipette. *Ceriodaphnia* that exhibit any response visible under the dissecting scope are considered living. Using the disposable transfer pipette, move the live adult *Ceriodaphnia* from the old cup to the new cup. If the animal has brooded record the number of neonates on the data sheet. If a dead brood is encountered, count the neonates to the best of your ability and place a letter “D” next to the number. If some portion of a brood is dead, note the number alive and dead with a slash between the numbers. When the adult dies and a brood remains, note the brood number, neonate number, and mark with an “X”. Save water from the old cup for water quality measurements. Feed survivors in the new cups 100 μL of YCT and 100 μL *Selenastrum*.

7.2 Measuring Water Quality in Test Containers

Measure temperature, dissolved oxygen, pH, ammonia, hardness, and conductivity in each sample at the beginning and end of the test. Sample the initial test solutions at the time of dilution preparation. Water quality should also be measured on old and new dilutions at the time of renewal. Renewal water quality parameters include dissolved oxygen, pH, conductivity, and temperature.

8.0 TERMINATING THE TOXICITY TEST

After 7 days of exposure final mortality and brood counts are made. Final water quality must be sampled at the termination of the test. Take the completed data sheet to the office for data entry and analysis. Notify the data analyst that the data has arrived. Make sure the data sheets are placed in the proper location and that the person keeping track of the data knows where it is.

9.0 DATA HANDLING AND TEST ACCEPTABILITY

Immediately after test termination, check the data sheet to determine whether dilution water and conductivity controls have acceptable survival (mean of > 90%). If not, notify the project officer without delay. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment.

This toxicity test procedure is considered acceptable if *Ceriodaphnia* survival in controls is greater than or equal to 80%. Sixty percent of surviving females must have produced 3 broods, and surviving females must have produced an average of 15 neonates. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment. Acceptable temperatures range from 25 ± 1°C; acceptable dissolved oxygen concentration is 60-100% saturation.

10.0 REFERENCES

U.S. Environmental Protection Agency. 1994. Short-term methods for estimating the chromic toxicity of effluents and receiving water to freshwater organisms. EPA-600-4-91-002. Office of Research and Development. Washington, DC.

11.0 TEST SUMMARY

Species:	*Ceriodaphnia dubia*
Test Duration:	7 days
Renewals:	Daily
Organism Source	In-house cultures or Toxscan, Watsonville

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Age of test organisms:	<24 hours
Test Conductivity:	<3000 μS
Test Temperature:	25 ± 1°C
Light intensity:	Ambient laboratory illumination 10-20 μE/μ^2/s
Photoperiod:	16 hour Light: 8 hour Dark; Replication: 5 replicates
Test Containers:	30-mL plastic cups
Test solution volume:	15-mL minimum
Loading:	1 neonate per beaker
Feeding:	In culture prior to test initiation and 100 μL YCT and *Selenastrum* daily after renewal
Water Quality:	pH, D.O. temp, conductivity, NH_3, hardness, ammonia
Reference Toxicant:	Copper Chloride ($CuCl_2$)
Stock Solution:	0.0268 g in 1 liter of distilled water (= 10,000 mg/L).
Dilutions:	0, 5.6, 10, 18, 32, 56 mg/L
Daily Monitoring:	Survival and Brood Size
Safety:	Wear protective clothing; read applicable MSDS, be familiar with the lab safety manual prior to testing.
Quality Control:	Fill out all data sheets completely. Be familiar with QA Project Plan prior to testing.
Acceptability Criteria:	Dilution Controls: >90% Surviving females: average 15 neonates Surviving females: 60% have 3 or more broods
Temperature range:	24° to 26°C.
Dissolved oxygen:	maximum 9.67 mg/L